

ULTRASTRUCTURAL STUDIES OF OOGENESIS AND SEXUAL MATURATION
IN FEMALE *CHLAMYS (AZUMAPECTEN) FARRERI FARRERI*
(JONES & PRESTON, 1904) (PTERIOMORPHIA: PECTINIDAE)
ON THE WESTERN COAST OF KOREA

Ee-Yung Chung

*School of Marine Life Science, Kunsan National University,
Gunsan 573-701, Korea; eychung@kunsan.ac.kr*

ABSTRACT

Ultrastructural studies of the development and degeneration of the oocytes and follicle cells in female *Chlamys (Azumapekten) farreri farreri* (Jones & Preston, 1904) are described for scallops collected from Daehuksando, Jeollanam-do, Korea. Vitellogenesis occurred by way of endogenous autosynthesis and exogenous heterosynthesis. Autosynthesis involved the combined activity of the Golgi complex, mitochondria, and rough endoplasmic reticulum, whereas heterosynthesis involved endocytotic incorporation of extraovarian precursors at the basal region of the early vitellogenic oocytes prior to the formation of the vitelline coat. Auxiliary cells were involved in the development of the previtellogenic and early vitellogenic oocytes and appear to play an integral role in vitellogenesis and oocyte degeneration by assimilating products originating from the degenerated oocytes, thus allowed the transfer of yolk precursors needed for vitellogenesis. Auxiliary cells presumably have a lysosomal system for breakdown products of oocyte degeneration. The reproductive cycle in females was classified into five stages: Stage I: early active stage (January to March), Stage II: late active stage (March to April), Stage III: ripe stage (April to August), Stage IV: partially spawned stage (June to August), and Stage V: spent/inactive stage (August to January). The spawning period continued from June to August, with a peak between July and August when the seawater temperature was exceeded 22°C. The percentage of first sexual maturity was 59.3% in individuals of 50.1–60.0 mm in shell height, and 100% in those > 70.1 mm in shell height.

Because harvesting clams less than 50.1 mm in shell height could potentially cause a drastic reduction in recruitment, a measure indicating a prohibitory fishing size should be enacted for adequate fisheries management.

Key words: *Chlamys (Azumapekten) farreri farreri*, oogenesis, auxiliary cell, first sexual maturation.

INTRODUCTION

The Jicon scallop, *Chlamys (Azumapekten) farreri farreri* (Jones & Preston, 1904) is a commercially important bivalve in East Asian countries, including Korea, Japan, and China. On the western coast of Korea, this species is mainly found in the gravel bed in the subtidal zone at depths up to 10 m (Yoo, 1976; Kwon et al., 1993; Min et al., 2004). Due to past overharvesting, it has been identified as a species requiring a more sustainable fishing regimen. For the propagation and management of this species, it is important that we fully understand the reproductive biology with regard to germ cell differentiation during oogenesis and sexual

maturation. Previously there have been many studies on reproduction in *C. farreri farreri*, including aspects of the reproductive cycle (Lioa et al., 1983; Yakovlev & Afeichuk, 1995), growth and spawning (Na et al., 1995; Kang & Zhang, 2000), experimental triploids and tetraploids (Yang et al., 1999a), its distribution and ecology (Whang & Kim, 1973), larval growth (Kuang et al., 1997; Yang et al., 1999b), and experimental aquaculture (Lim et al., 1995; Sun et al., 1996, 1997).

Despite this, there are still significant gaps in our knowledge regarding its reproductive biology. Above all, studies on the development and degeneration of the oocytes and auxiliary cells during oogenesis of *C. farrerii farreri* are

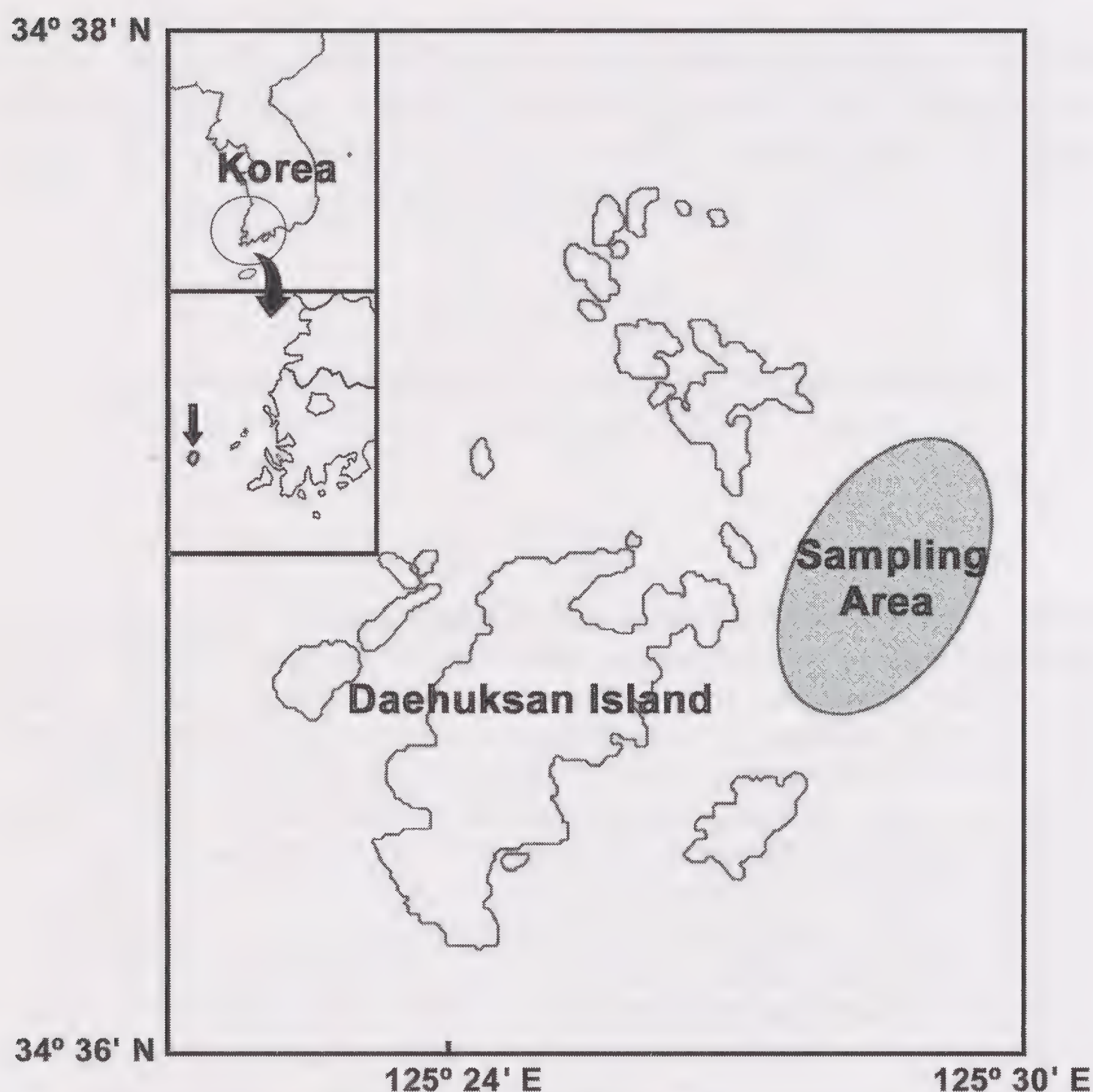


FIG. 1. Map of the sampling area.

required to understand this animal's reproductive biology. In the majority of bivalve species, the ovaries contain auxiliary cells, a kind of accessory cell, that play a role in the storage, mobilization, and synthesis of yolk precursors during oogenesis (Wourms, 1987). More specifically, oocyte degeneration, which is known as atresia, is a commonly observed phenomenon in most bivalve species. In bivalves, the products of lysis material created by the auxiliary cells act as sources of metabolites that can be rapidly mobilized by the organism (Pipe, 1987; Dorange et al., 1989; Le Pennec et al., 1991; Gaulejac et al., 1995). Above all, the functions of the auxiliary cells in the resorption of the lysis products of atretic oocytes of this species should be investigated in further detail.

Understanding of the reproductive cycle and spawning period of this species will provide information needed for the determination of the size at first reproduction and the recruitment period. Additional information on the shell size attained when 50% of the individuals reach first sexual maturity can determine a prohibitory size for adequate natural resource man-

agement. Therefore, the purpose of this paper is to describe vitellogenesis during oogenesis, the reproductive cycle, and the size at first sexual maturity in *C. farreri farreri*, using cytological, histological, and morphometric procedures. Results will be useful for improved fisheries management of this species.

MATERIALS AND METHODS

Sampling

Jicon scallops were collected monthly by dredge in the gravel bed in the subtidal zone at depths up to 10 m off Daehuksando Island, Jeollanam-do, Korea (Fig. 1), from January 2003 to December 2004.

Histology (Light Microscopy)

For light microscopic examination of histological preparations, female ovarian tissues were removed from animals and preserved in Bouin's fixative for 24 h, then washed with run-

ning tap water for 24 h. Tissues were then dehydrated in alcohol and embedded in paraffin molds. Embedded tissues were sectioned at 5–7 μm thickness using a rotary microtome. Sections were mounted on glass slides, stained with Hansen hematoxylin – 0.5% eosin, and examined using a light microscope. These stained sections were analyzed to (1) describe the ovarian cycle, and (2) determine the size at which sexual maturity is attained, the methods described below.

Ovarian Cycle by Light Microscopical Observation

To describe the ovarian cycle and to identify the spawning period, a total of 731 ovarian histological preparations were made from scallops of 50.1–94.7 mm shell height.

Ultrastructure of Germ Cells and Auxiliary Cells During Oogenesis and Oocyte Degeneration

A total of 95 females was used for ultrastructural study of germ cells and auxiliary cells by electron microscopy. For transmission electron microscopy, excised samples of gonads were cut into small pieces and fixed immediately in 2.5% paraformaldehyde-glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4°C. After prefixation, the specimens were washed several times in the buffer solution and then postfixed in 1% osmium tetroxide solution in 0.2 M phosphate buffer (pH 7.4) for 1 h at 4°C. Specimens then were dehydrated in increasing concentrations of ethanol, cleared in propylene oxide, and embedded in an Epon-Araldite mixture (Epon-812). Ultrathin sections of Epon-embedded specimens were cut to a thickness of 80–100 nm with a LKB ultramicrotome. The sections were mounted on collodion-coated copper grids, double stained with uranyl acetate, followed by lead citrate, and observed under a JEM 100 CX-2 (80 kv) electron microscope.

Size at First Sexual Maturity by Light Microscopical Observation

For determination of the size at 50% of first sexual maturity, a total of 208 ovarian histological preparations (30.4–94.7 mm shell height) were examined the size at 50% first sexual maturity (= biological minimum size) by histological observations from May to October 2003. The percentage (%) of first sexual maturity = No. of mature individuals $\times$ 100/No. of total individuals investigated.

RESULTS

Position and Morphology of the Ovary

Chlamys farreri farreri is dioecious. The ovary is conical or crescent shaped, and it is separated from the digestive diverticula and the adductor muscle. It is located from the ventral region of the visceral mass to the adductor muscle. The ovary is a diffuse organ composed of highly branching follicles (acini) in which germ cells develop.

As ovarian maturation progressed, the ovary encircled part of the adductor muscle, and the external color of the ovary became pink (the testis being milky white or light yellow). Therefore, sex could be easily determined from external features. At this time, mature oocytes readily emerged when the ovary was slightly scratched. After spawning, the ovary degenerated, and then the sexes became difficult to distinguish.

Annual Reproductive Cycle with Ovarian Developmental Stages

Based on electron microscopical and histological observations of the germ cells and other surrounding cells (auxiliary cells), the gonadal phases were classified into five successive stages (Fig. 2). The stages and the criteria used in defining them are as follows:

Stage 1 (early active stage): Oogonia and previtellogenic oocytes propagate along the follicular wall of the ovary. The oogonia were about 10–11 μm in diameter, and the previtellogenic oocytes 16–20 μm in diameter. The lumina of the oogenic follicles were empty during the early active stage, although the auxiliary cells, which were attached to the previtellogenic oocyte, appeared in the oogenic follicle at this stage (Fig. 3A). In 2003 and 2004, female individuals in stage I (early active stage) appeared from January to March when seawater temperatures were about 10°C.

Stage II (late active stage): At a size of 30–40 μm in diameter, each early vitellogenic oocyte formed an egg-stalk connected to the follicular wall (germinal epithelium), and the auxiliary cells, which were attached to the oocyte, appeared in the lumen of the follicle.

At a diameter of 40–50 μm , each late vitellogenic oocyte had a large germinal vesicle and an egg-stalk attached to the fol-

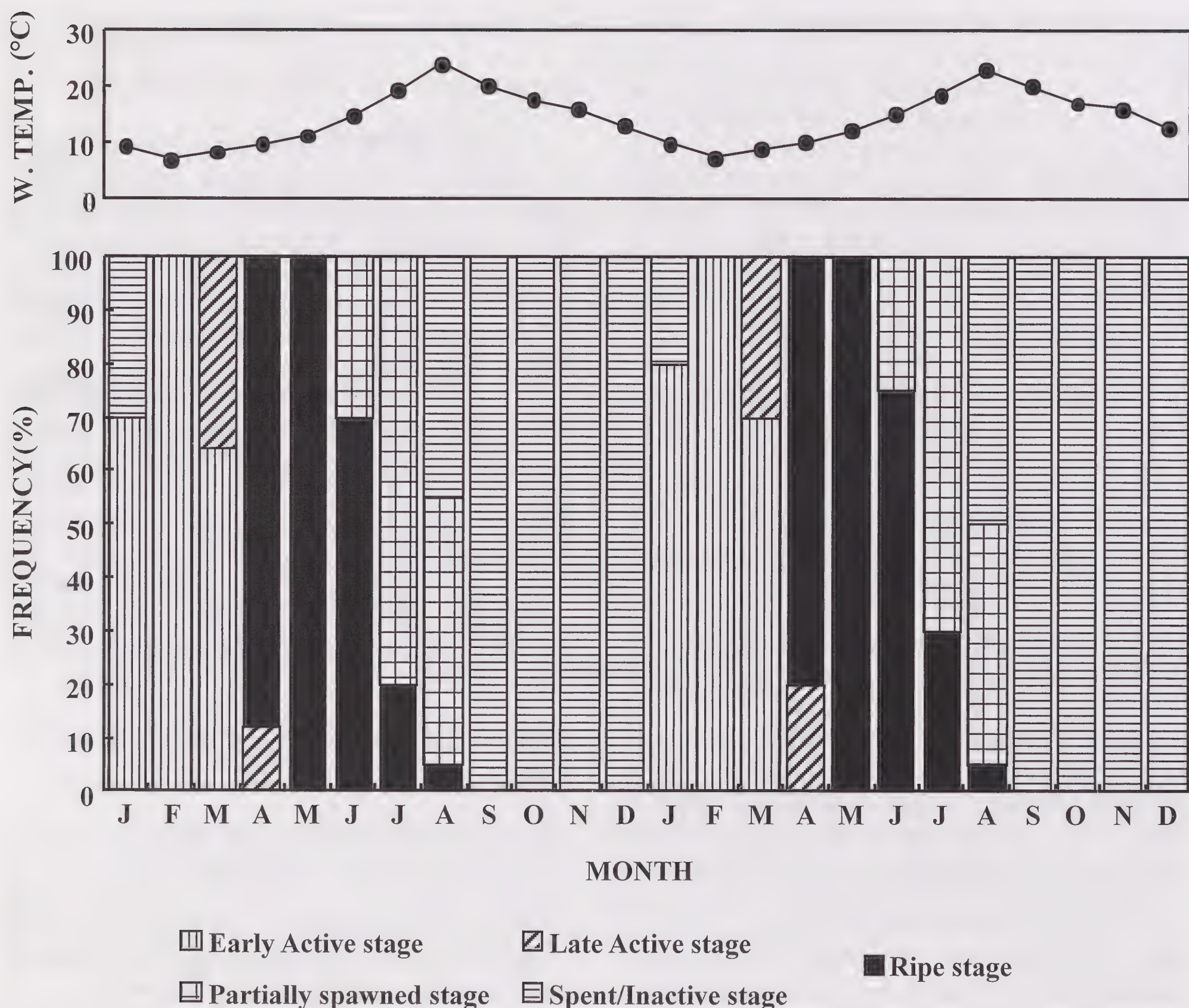


FIG. 2. Frequency of gonadal phases in female *Chlamys farreri farreri* compared with mean seawater temperatures from January 2003 to December 2004.

licular wall (Fig. 3B). In 2003 and 2004, female individuals in stage II (late active stage) were found from March to April when seawater temperatures were relatively low (7–13°C).

Stage III (ripe stage): The majority of maturing oocytes grew to 50–60 µm in diameter, becoming round or oval in shape, and were located in the center of the lumen. Each ripe ovum (60–70 µm in diameter) was surrounded by a gelatinous membrane and its cytoplasm was filled with a large number of yolk granules (Fig. 3C). At this time, the auxiliary cells detached from the mature oocyte. In 2003 and 2004, female individuals in stage III (ripe stage) appeared from April through August when seawater temperature gradually increased over 15°C.

Stage IV (partially spawned stage): Most ripe ova were discharged from the oogenic follicles, although a few undischarged mature oocytes as well as vitellogenic oocytes remained (Fig. 3D). In 2003 and 2004, female individuals in stage IV (partially spawned stage) were found from June to August, and the main spawning occurred between July and August when seawater temperatures were higher than 22°C.

Stage V (spent / inactive stage): After spawning, each follicle contracted and degenerated, and the undischarged oocytes in the lumen of the follicle underwent cytolysis. The products of gamete atresia were resorbed (Fig. 3E). Thereafter, a rearrangement of the connective tissues was observed. Occasion-

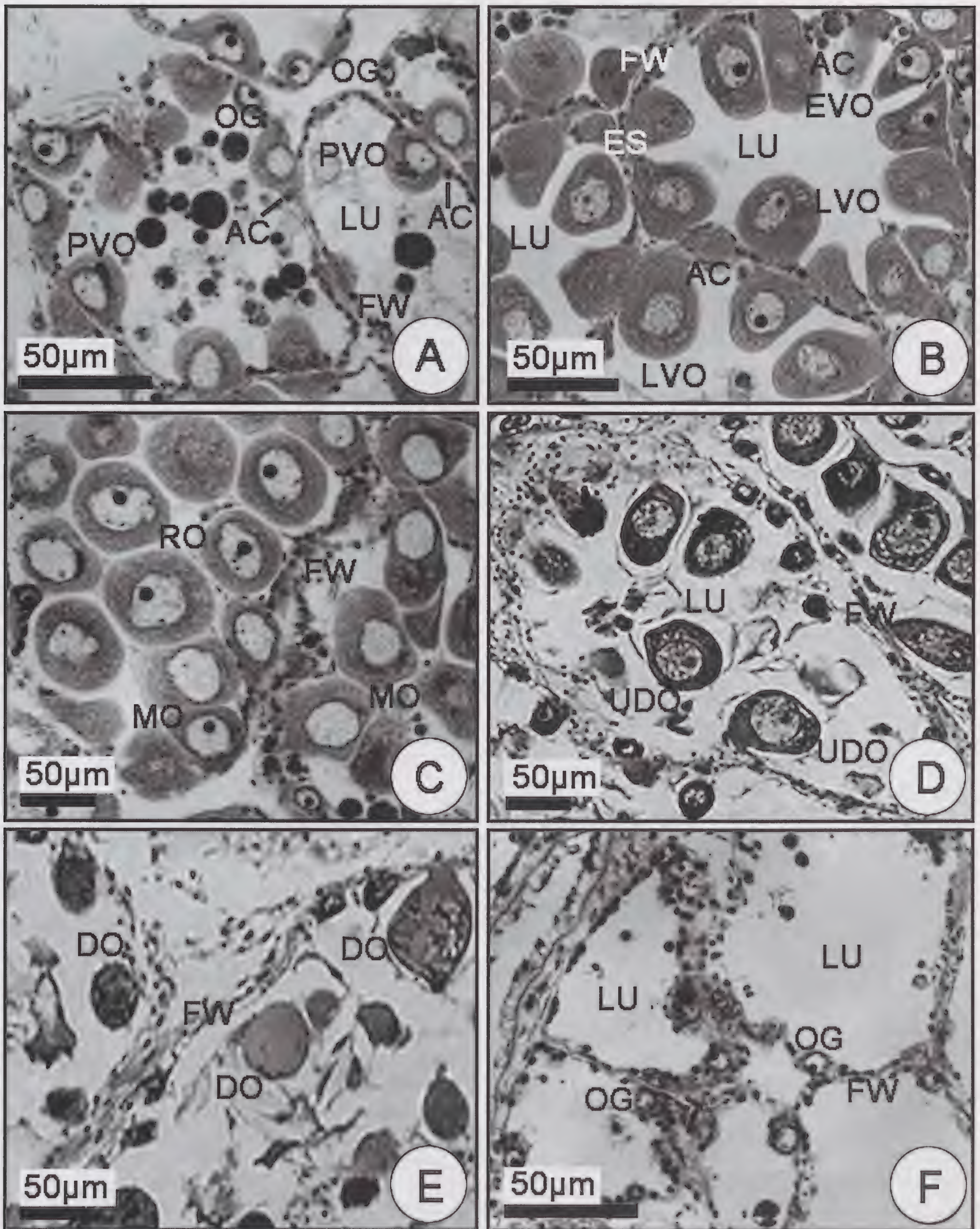


FIG. 3. Photomicrographs of oogenic follicles in various gonadal phases in female *Chlamys farreri*. A: Early active stage; B: Late active stage; C: Ripe stage; D: Partially spawned stage; E: Spent stage; F: Inactive stage. Abbreviations: AC = auxiliary cell; DO = degenerated oocyte; ES = egg stalk; EVO = early vitellogenic oocyte; FW = follicular wall; LU = lumen; LVO = late vitellogenic oocyte; MO = maturing oocyte; OG = oogonium; PVO = previtellogenic oocyte; RO = ripe ovum; UDO = undischarged oocyte.

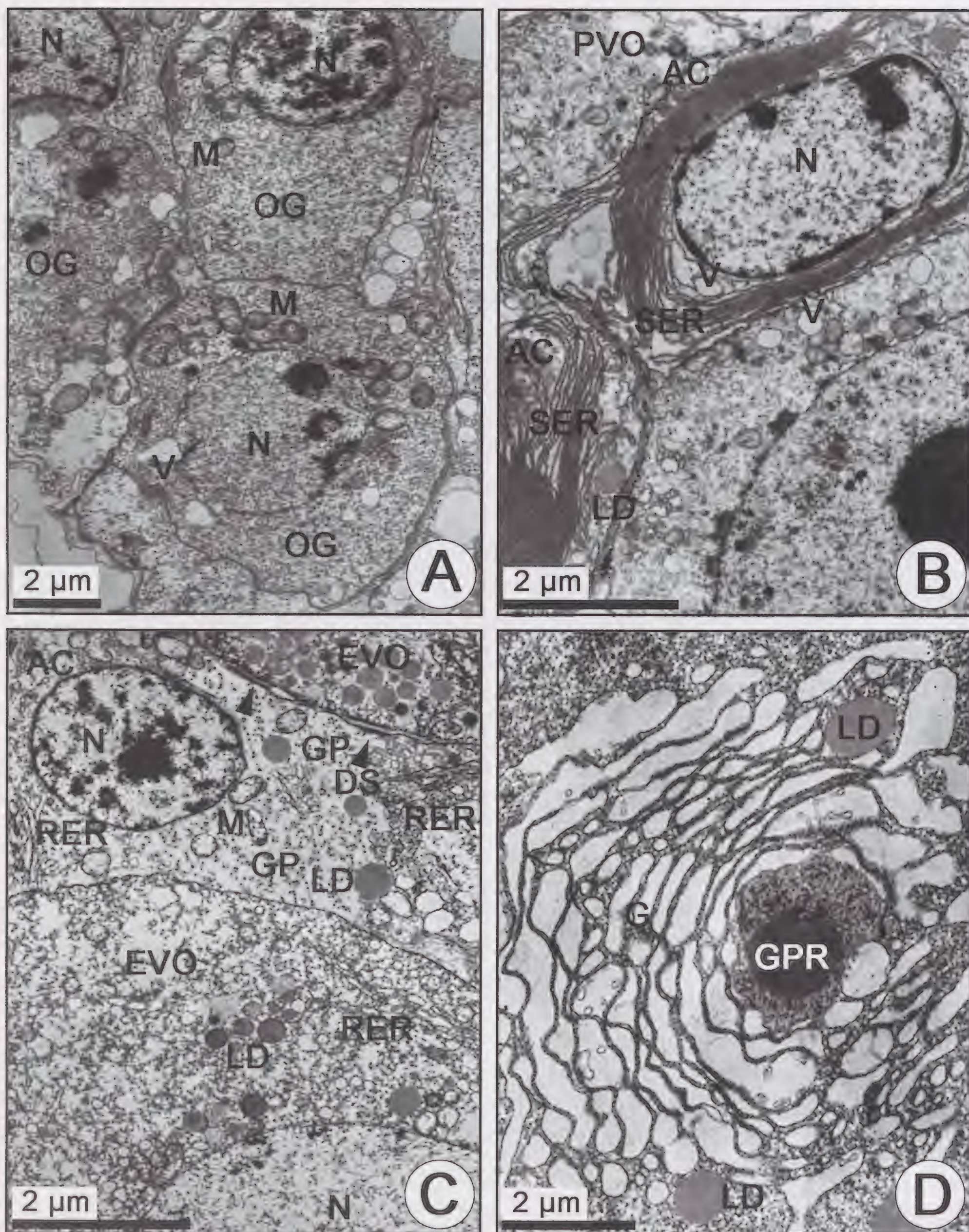


FIG. 4. Electron micrographs of oogenesis in female *Chlamys farreri farreri*. A: Oogonia, with a large nucleus, several mitochondria, and vacuoles in the cytoplasm; B: Previtellogenic oocyte, with a nucleolus in the nucleus, the mitochondria, rough endoplasmic reticulum, and an attached auxiliary cell containing a nucleus, mitochondria, and rough endoplasmic reticulum; C: Early vitellogenic oocyte and desmosome (arrow head), with a nucleus and several lipid droplets, and attached auxiliary cells containing lipid droplets and glycogen particles; D: Early vitellogenic oocyte, with the Golgi products in the Golgi complex near lipid droplets. Abbreviations: AC = auxiliary cell; DS = desmosome; EVO = early vitellogenic oocyte; G = Golgi complex; GP = glycogen particle; GPR = Golgi product; LD = lipid droplet; M, mitochondrion; N = nucleus; OG = oogonium; PVO = previtellogenic oocyte; RER = rough endoplasmic reticulum; SER = smooth endoplasmic reticulum; V = vacuole.

ally, a few oogonia were present on the follicular walls in this stage (Fig. 3F). In 2003 and 2004, female individuals in stage (spent/inactive stage) appeared from August through January.

Ultrastructure of Germ Cells and Auxiliary Cells during Oogenesis and Oocyte Degeneration

Based on electron microscopical observations, four distinct phases of oogenesis were distinguished in germ cells, that is, oogonia, previtellogenic oocytes, vitellogenic oocytes, and mature oocytes (Eckelbarger & Davis, 1996).

Oogonia: The oogonia, which measured from 10–11 μm in diameter, were round in shape. Each possessed a large ovoid nucleus, in which the chromatin was reticular and marginal. The oogonia, which multiply within the follicular wall (germinal epithelium), were single or formed a cluster in the follicle. Several mitochondria, the Golgi complex, and vacuoles appeared in the cytoplasm of oogonia (Fig. 4A).

Previtellogenic Oocytes: The oogonia develop into previtellogenic oocytes. At the beginning of cytoplasmic growth of the previtellogenic oocyte, several mitochondria and vacuoles were concentrated around the nucleus. At this time, the auxiliary cells initially appeared close to the oocyte, and thereafter, progressively surrounded the oocyte. Close contact was maintained with the auxiliary cell. Near the adherence zone, vacuoles were visible in the cytoplasm of the auxiliary cells (Fig. 4B).

Vitellogenic Oocytes: In the early vitellogenic oocyte, lipid droplets, mitochondria, and endoplasmic reticulum were usually present in the perinuclear region. Desmosomes were found in the attached parts of the early vitellogenic oocyte connected to the auxiliary cell. In particular, the mitochondria, lipid droplets, and glycogen particles appeared in the cytoplasm of the auxiliary cells (Fig. 4C). During early oogenesis, lipid droplets appeared near the Golgi product formed by the Golgi complex in the cytoplasm of the early vitellogenic oocyte (Fig. 4D), and were also found between the mitochondria and well-developed rough endoplasmic reticulum in the cytoplasm of the early vitellogenic oocyte (Fig. 5A). At this time, coated vesicles,

resulting from endocytosis appeared at the basal region of the early vitellogenic oocyte. The uptake of nutritive material in the coated vesicles formed by receptor-mediated endocytosis appeared through the formation of coated endocytotic pits on the oolemma (Fig. 5B). At the same time yolk granules clearly appeared among the mitochondria, lipid droplets, and the endoplasmic reticulum at the cortical region (Fig. 5C). In the mid-vitellogenic oocyte, multivesicular bodies, which were formed by modified mitochondrial cristae, appeared between the nuclear envelope and the cortical region (Fig. 5D). Thereafter, yolk granules and lipid droplets filled the cytoplasm of the late vitellogenic oocyte, whereas the auxiliary cells gradually lost their intimate association with the late vitellogenic oocyte surface. The cytoplasm of the auxiliary cells, which was detached from the oocyte, was filled with vacuoles and myelin figure (Fig. 6A). After yolk granules were mixed with coated vesicles (by endocytosis) and multivesicular bodies, and then small yolk granules were formed near the cortical region of the late vitellogenic oocyte (Fig. 6B). In the late stages of oogenesis, large yolk granules were formed by a combination of small yolk granules (Fig. 6C).

Mature Oocytes: In the mature oocyte, small yolk granules were continuously combined and became larger mature yolk granules in the cytoplasm. A mature yolk granule is composed of three components: (1) crystalline core, (2) electron lucent cortex, and (3) a limiting membrane (Fig. 6D).

Oocyte Degeneration: The degenerating oocytes appeared slightly irregular or polyhedral near the auxiliary cells and were deformed by compression in the follicle. A number of vacuoles, degenerating yolk granules a few phagosomes (lysosomes), and lipid droplets appeared in the cytoplasm of degenerating oocyte. At this stage, especially in the auxiliary cells, a few phagosomes (lysosomes) and a number of vacuoles, and a small number of lipid droplets appeared in the cytoplasm of the auxiliary cell, whereas glycogen particles decreased in the cytoplasm of the auxiliary cells, which were attached to the degenerating oocyte (Fig. 7A). During the gradual disintegration of the oocytes, the endoplasmic reticulum was specifically involved in the degenerative process. The smooth or rough endoplasmic reticulum became distended,

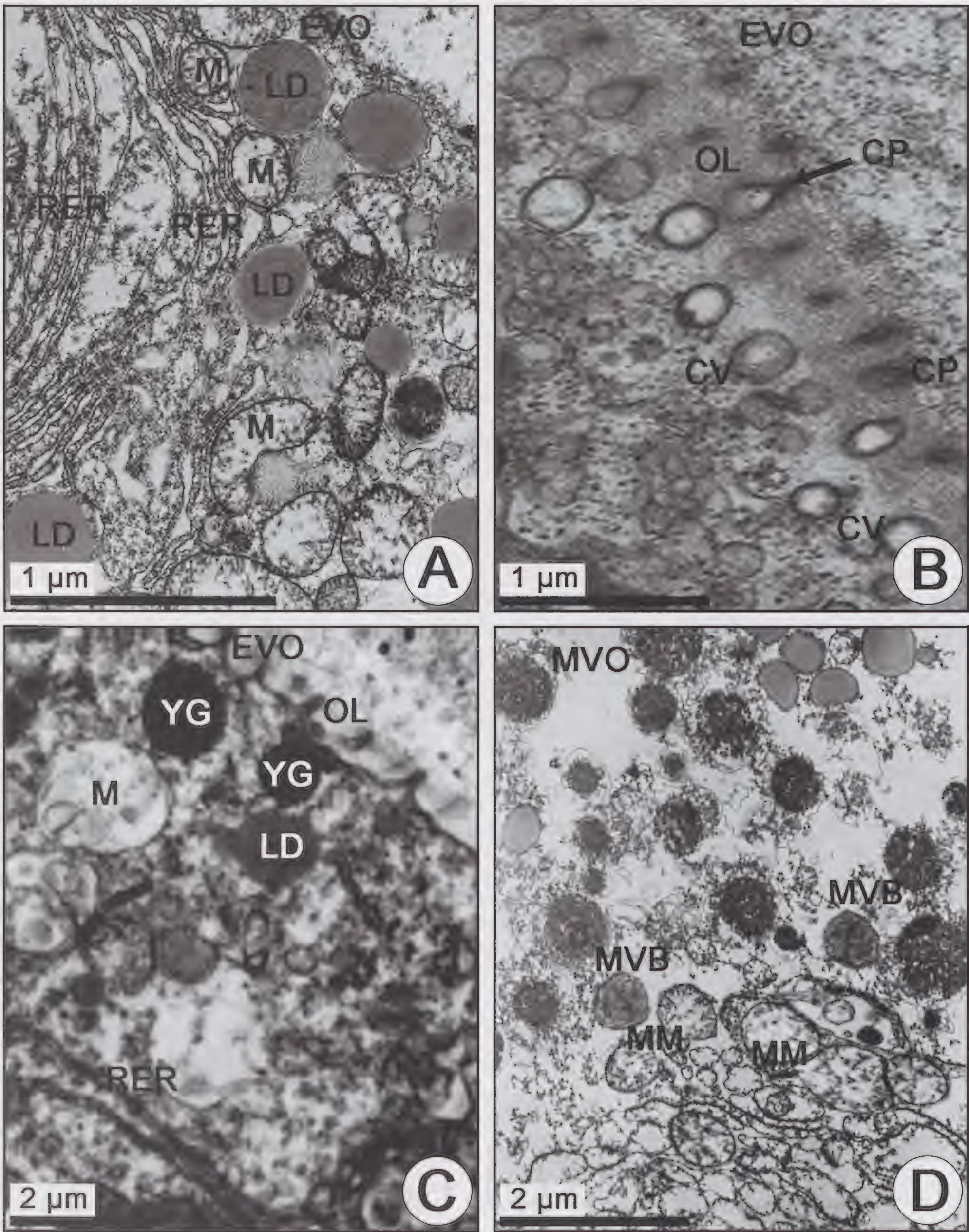


FIG. 5. Electron micrographs of oogenesis in female *Chlamys farreri farreri*. A: Early vitellogenic oocyte, with lipid droplets between well-developed rough endoplasmic reticulum and the mitochondria; B: Early vitellogenic oocyte, with coated vesicles at the basal region through the coated endocytotic pits (upper left) formed by endocytosis; C: Early vitellogenic oocyte, with yolk precursors among rough endoplasmic reticulum, mitochondria, and lipid droplets; D: Mid-vitellogenic oocyte, with multivesicular bodies formed by the modified mitochondria. Abbreviations: CP = coated pit; CV = coated vesicle; EVO = early vitellogenic oocyte; LD = lipid droplet; M = mitochondrion; MM = modified mitochondrion; MVB = multivesicular body; MVO = mid-vitellogenic oocyte; OL = oolemma; RER = rough endoplasmic reticulum; YG = yolk granule.

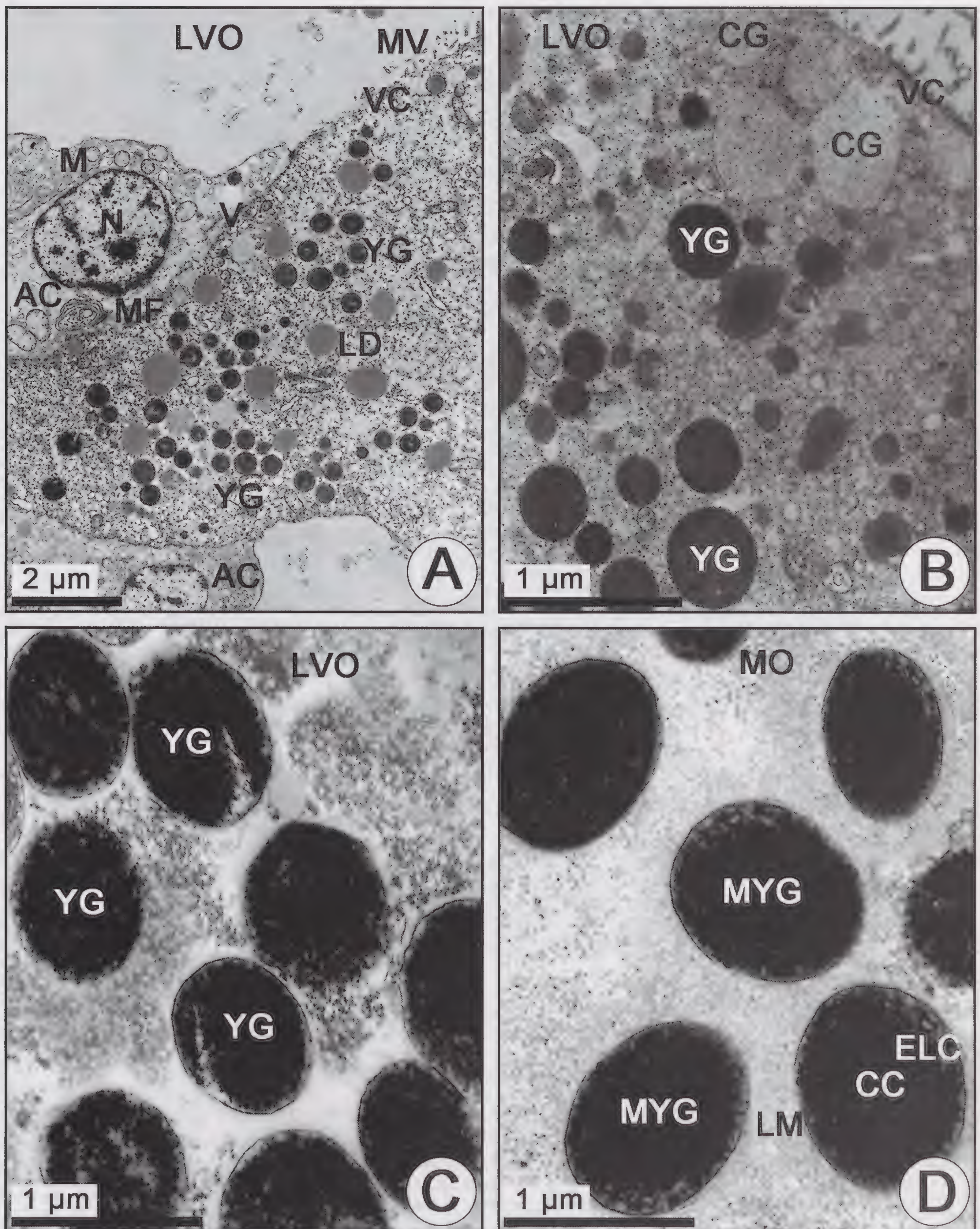


FIG. 6. Electron micrographs of oogenesis in female *Chlamys farreri farreri*. A: Late vitellogenic oocyte, with a number of yolk precursors, lipid droplets, microvilli on the vitellogenic coat, and auxiliary cells containing lipid droplets, vacuoles, and a myelin figure; B: Late vitellogenic oocyte, with cortical granules near the vitelline coat, and proteinaceous yolk granules; C: Late vitellogenic oocyte, with proteinaceous yolk granules and a number of immature yolk granules; D: Mature oocyte, with a number of mature yolk granules each composed of three parts: 1) crystalline core, 2) electron lucent cortex, and 3) a limiting membrane. Abbreviations: AC = auxiliary cell; CC = crystalline core; CG = cortical granule; ELC = electron lucent cortex; LD = lipid droplet; LM = limiting membrane; LVO = late vitellogenic oocyte; M = mitochondrion; MF = myelin figure; MO = mature oocyte; MV = microvillus; MYG = mature yolk granule; N = nucleus; PHA = phagosome; V = vacuole; VC = vitelline coat; YG = yolk granule.

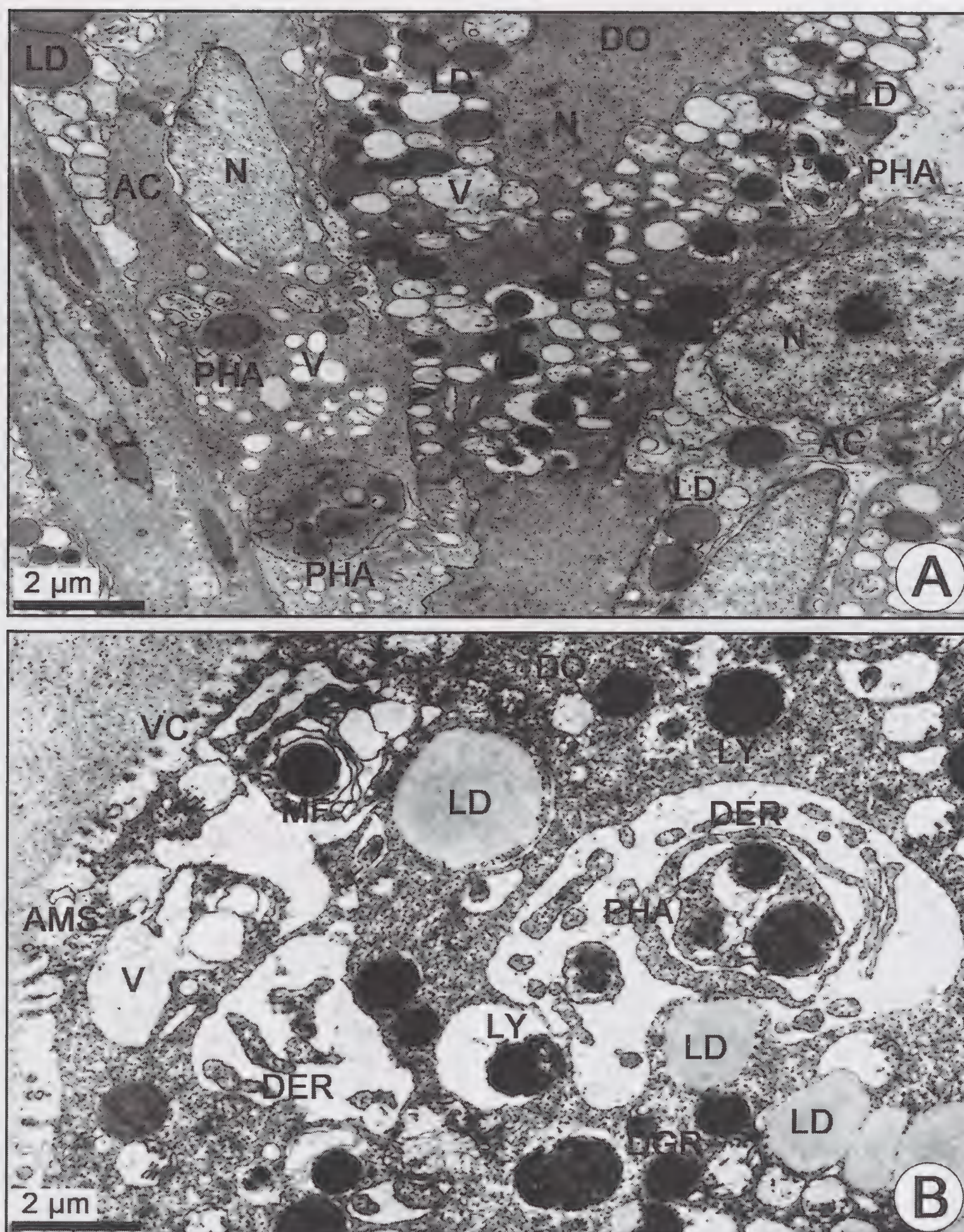


FIG. 7. Electron micrographs of degenerated oocytes with auxiliary cells in female *Chlamys farerri. farerri*. A: Degenerating oocyte, with degenerating yolk granules, phagosomes by lysosome, and the auxiliary cells containing lipid droplets and various phagosomes and vacuoles in the cytoplasm; B: Degenerated oocyte, with distended endoplasmic reticulum, vacuoles, degenerated granules, myelin figure organelle, phagosomes (lysosomes) near the lipid droplets, and abnormal vitelline coat. Abbreviations: AC = auxiliary cell; AMS = abnormal microvillus structure; DER = distended endoplasmic reticulum; DGR = degenerating granule; DO = degenerated oocyte; LD = lipid droplet; LM = limiting membrane; LY = lysosome; M = mitochondrion; MF = myelin figure; N = nucleus; PHA = phagosome; V = vacuole; VC = vitelline coat.

which led to vacuolation of the ooplasm. At this time, abnormal microvillus structure appeared on the vitelline coat of the degenerated oocyte. Mitochondria and yolk granules disintegrated in the ooplasm, and lysis was initiated at the cell periphery, several vacu-

oles and numerous heterogenous, dense granules that appear similar to phagosomes (lysosomes) were present in the ooplasm. In particular, many disintegrated granules with myelin figure and phagosomes were visible at the periphery of the oocyte (Fig. 7B).

TABLE 1. Shell height at first sexual maturity in female *Chlamys (Azumapecten) farreri farreri* from May to October 2003. Ind. = Individual.

Shell height (mm)	Number of Individuals by Gonadal Stage*					Total Ind.	Mature (%)
	EA	LA	RI	PS	SP/IA		
30.4–40.0	24					24	0
40.1–50.0	15	6	5	2		28	39.3
50.1–60.0	11	4	8	4		27	59.3
60.1–70.0	2	2	17	13	4	38	94.7
70.1–80.0			16	12	4	32	100.0
80.1–90.0			15	9	6	30	100.0
90.1–94.7			14	10	5	29	100.0
Total						208	

*Gonadal stage: EA = Early Active Stage; LA = Late Active Stage; RI = Ripe Stage; PS = Partially Spawned Stage; SP/IA = Spent/Inactive Stage.

Size at First Sexual Maturity

As shown in Table 1, it was found that gonadal development of smaller individuals ranging from 30.4–40.0 mm in shell height were in the early active stage, characterized by a small number of oogonia and the previtellogenic oocytes present. During the period between June and August, when spawning was observed among older individuals. However, younger animals (30.4 to 40.0 mm in shell height) had a small number of oogonia and a number of previtellogenic oocytes were present in the follicles of the ovary. It is supposed that their sizes at sexual maturity could not have been reached until late August when spawning was completed. In addition, the percentage of first sexual maturity of female scallops ranging from 40.1–50.0 mm shell height is 39.3%, but those individuals were in a variety of gonadal stages during the breeding season. In contrast, all individuals of shell height greater than 70.1 mm displayed ripe, partially spawned, or spent/inactive stages. Accordingly, it is assumed that most individuals can reach full maturity by late August if they are larger 70.1 mm in shell height at that time.

DISCUSSION

Gamete Differentiation and Vitellogenesis

Although many authors suggested the formation of lipid droplets in several species, no clear morphological evidence has been shown for the processes involved in lipid droplet for-

mation thus far (Pipe, 1987; Dorange & Le Pennec, 1989; Gaulejac et al., 1995). In our present study, however, lipid droplets appeared among the Golgi complex, well-developed endoplasmic reticulum, and mitochondria in the early vitellogenic oocytes. Therefore, it is assumed that they may be involved in the formation of lipid droplets (Chung & Ryou, 2000; Chung et al., 2002, 2005, 2006; Chung, 2007). Vitellogenesis showed a possibility of auto-synthetic and heterosynthetic yolk formation. The yolk granules originate in the cortical regions of the oocyte, and then fill the entire ooplasm of the oocyte. However, the sizes of yolk granules varies in different regions of the egg. Therefore, various cell organelles, in particular the Golgi complex, endoplasmic reticulum and mitochondria are thought to be involved in endogenous formation of yolk granules in the cytoplasm (Pipe, 1987; Dorange & Le Pennec, 1989; Gaulejac et al., 1995; Chung et al., 2005; Chung, 2007), while the uptake of nutritive material in the coated vesicle formed by receptor-mediated endocytosis appeared through the formation of coated endocytotic pits on the oolemma (Chung, 2007). From these observations, it is assumed that vitellogenesis in *C. farreri farreri* occurs by way of endogenous autosynthesis and exogenous heterosynthesis. Autosynthesis involves the combined activity of the Golgi complex, mitochondria, and rough endoplasmic reticulum; heterosynthesis occurs as the incorporation of extraovarian precursors into oocytes by endocytosis, which involves the basal region of the early vitellogenic oocytes prior to the formation of the vitelline coat.

Functions of the Auxiliary Cells

In stage I (early active stage), the auxiliary cells at the periphery of the oogenic follicle (or acinus) initially appears close to the previtellogenic oocyte, and thereafter, progressively surrounds a part of the oocyte. At this stage, a small number of vacuoles were visible in the cytoplasm of the auxiliary cells near the adherence zone. The attached auxiliary cells also showed cytological modifications as their cytoplasmic volume increased in *Crassostrea virginica* (Eckelbarger & Davis, 1996) and *Mytilus edulis* (Pipe, 1987).

In the present study of *C. farreri farreri*, several auxiliary cells attached to previtellogenic and early vitellogenic oocytes during the early stages of oogenesis, but, most auxiliary cells, detached from the mid- or late vitellogenic oocytes; only a few cells appeared near the stalk region of the oocyte. At the adherence zone between of the auxiliary cells and vitellogenic oocytes, lipid droplets and a number of vacuoles, and the myelin figures appeared in the cytoplasm of the auxiliary cells, which is indicative of membrane breakdown (Pipe, 1987). Because the auxiliary cells are abundant on the oocyte in the early stages of oogenesis and gradually detach from the vitellogenic oocyte, it is assumed that auxiliary cells function as nutritive cells in the early formation and development of the oocytes. In the scallop, *Pecten maximus*, Dorange & Le Pennec (1989) proposed that the "auxiliary cells" of *P. maximus* might play a role in oocyte nutrition and vitelline envelope formation partially due to the presence of extensive RER cisternae. RER cisternae have been reported in many bivalve follicle cells (Gaulejac et al., 1995), while the follicle cells of *Crassostrea gigas* were reported to contain smooth endoplasmic reticulum and desmosomes (Eckelbarger & Davis, 1996). In this study, smooth endoplasmic reticulum and desmosomes between oocyte and auxiliary cells could be seen. Therefore, our results coincide with the report of Eckelbarger & Davis (1996), and nutrients in the auxiliary cells presumably transport to the oocyte by the desmosome.

Pipe (1987) reported that endocytotic figures appeared between vitellogenic oocytes and the auxiliary cells, indicating a transfer nutrients in *Mytilus edulis*. In the present study, endocytotic-coated vesicles, indicating a transfer nutrients, appeared between the auxiliary cells and the vitellogenic oocytes. Therefore, the results mentioned above showed a similar phenomenon to those reported by Pipe (1987).

Oocyte Degeneration and the Functions of the Auxiliary Cells

In the present study, the characteristics of a functional role of lysosomes and a number of degenerated yolk granules containing a few myelin figures appeared in the ooplasm of the degenerated oocytes in *C. farreri farreri*. At the same time, several phagosomes (lysosomes) near the lipid droplets appeared in the cytoplasm of the auxiliary cells, that were attached to the degenerated oocytes. In particular, morphologically similar phagosomes (lysosomes), which were easily observed in the cytoplasm of degenerated oocytes, also appeared in the auxiliary cells. Thus, the auxiliary cells appear to play an integral role in vitellogenesis and oocyte degeneration. During the period of oocyte degeneration, the auxiliary cells function in phagocytosis and intracellular digestion of products originating from oocyte degeneration; these cells might also have a function associated with the induction of oocyte degeneration, and it is assumed that they are also active in the resorption of phagosomes (lysosomes) from the degenerated oocyte, because lipid droplets and degenerating phagosomes appeared in the auxiliary cells. In this study, the number of lipid granules gradually increased in auxiliary cells during gametogenesis; this function can permit a transfer of yolk precursors necessary for vitellogenesis and allow for the accumulation of reserves in the cytoplasm as glycogen and lipids, which can be employed by vitellogenic oocytes (Gaulejac et al., 1995). Therefore, it is assumed that the auxiliary cells, that are attached to degenerated oocytes, presumably have a lysosomal system for breakdown of ingested material, and they might be involved in the induction of oocyte degeneration, and might also resorb various phagosomes (lysosomes) in the cytoplasm for nutrient storage, such as lipid droplets, during oocyte degeneration, as seen in *Meretrix lusoria* (Chung, 2007).

Fate of the Gametes

Regarding reproductive energy allocated to the production of gametes, some authors (Morvan & Ansell, 1988) stated that continuous production and resorption of gametes can be regarded as an adaptation to environmental temperature and food availability. If the energy allocated to the production of gametes is too large, nutritive reserves might not be sufficient to allow all eggs to reach the critical size and maturity for spawning and fertilization. In this case, the products of gamete atresia can

be resorbed and the energy reallocated to still-developing oocytes or used for other metabolic purposes by marine mollusks (Dorange & Le Pennec, 1989; Mortavkine & Varaksine, 1989).

In *Mytilus edulis*, after spawning, gamete resorption is common in the acini of the ovary. It is supposed that *Mytilus edulis* resorbs gametes in follicles to utilize the high nutritive reserves in developing oocytes for other metabolic activities (Pipe, 1987) as observed in other bivalves (Dorange & Le Pennec, 1989; Motavkine & Veraksine, 1989). Therefore, it is assumed that *C. farreri farreri* has a similar reproductive mechanism to resorb and utilize high nutritive substances rather than releasing non-viable gametes.

Size at First Sexual Maturity

The percentage of first sexual maturity of individuals of 50.1 to 60.0 mm in shell height, in the late active, ripe, and partially spawned stages, was over 50%, and was 100% in those over 70.1 mm in shell height in the late active, ripe, partially spawned, and spent/inactive stages. Accordingly, it is likely that most individuals will have reached maturity by late August if larger than 70.1 mm in shell height. This means that larger individuals can reach maturity earlier than smaller individuals, indicating that harvesting scallops less than 50.1 mm in shell height could cause a drastic reduction in recruitment. Accordingly, an enforced, minimum legal fishing size should prove adequate for fisheries management of this species. Thus, in particular, information on the size at 50% of sexual maturity is very important, and can determine a prohibitory fishing size for adequate natural resources management through determination of size at 50% first sexual maturity. However, further detailed studies on age determination of this species should be carried out to better understand the population dynamics.

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LITERATURE CITED

- CHUNG, E. Y., 2007, Oogenesis and sexual maturation in *Meretrix lusoria* (Röding, 1798) (Bivalvia: Veneridae) in western Korea. *Journal of Shellfish Research*, 26: 1–10.
- CHUNG, E. Y., S. Y. KIM, K. H. PARK & G. M. PARK, 2002, Sexual maturation, spawning, and deposition of the egg capsules of the female purple shell, *Rapana venosa* (Gastropoda: Muricidae). *Malacologia*, 44: 241–257.
- CHUNG, E. Y., S. Y. KIM, G. M. PARK & J. M. YOON, 2006, Germ cell differentiation and sexual maturation of the female *Neptunea (Barbitonia) arthritica cumingii* (Crosse, 1862) (Gastropoda: Buccinidae). *Malacologia*, 48: 65–76.
- CHUNG, E. Y., Y. J. PARK, J. Y. LEE & D. K. RYU, 2005, Germ cell differentiation and sexual maturation of the hanging cultured female scallop *Patinopecten yessoensis* on the east coast of Korea. *Journal of Shellfish Research*, 24: 913–921.
- CHUNG, E. Y. & D. K. RYOU, 2000, Gametogenesis and sexual maturation of the surf clam *Macra veneriformis* on the west coast of Korea. *Malacologia*, 42: 149–163.
- DORANGE, G. & M. LE PENNEC, 1989, Ultrastructural study of oogenesis and oocytic degeneration in *Pecten maximus* from the Bay of St. Briey. *Marine Biology*, 103: 339–348.
- DORANGE, G., Y. M. PAULET & M. LE PENNEC, 1989, Étude cytologique de la partie femelle de la gonade de *Pecten maximus* récolte en baie de Saint-Brieux. 2. Ovogenese et lyse ovocytaire. *Halotiss*, 19: 299–314.
- ECKELBARGER, K. J. & C. V. DAVIS, 1996, Ultrastructure of the gonad and gametogenesis in the eastern oyster, *Crassostrea virginica*. 1. Ovary and oogenesis. *Marine Biology*, 127: 79–87.
- GAULEJAC, De. B., M. HENRY & N. VICENTE, 1995, An ultrastructural study of gametogenesis of the marine bivalve *Pinna nobilis* (Linnaeus 1758). 1. Oogenesis. *Journal of Molluscan Studies*, 61: 375–392.
- KANG, T. G. & C. I. ZHANG, 2000, A study on the growth and spawning of Korean scallop (*Chlamys farreri*) around Wando, Korea. *Journal of the Korean Fisheries Society*, 36: 210–221 [in Korean].
- KUANG, S., H. SUN, F. LI & J. FANG, 1997, Feeding and growth of scallop *Chlamys farreri* before and after spawning. *Marine Fisheries Research of China*, 17: 80–86.
- KWON, O. K., G. M. PARK & J. S. LEE, 1993, *Coloured shells of Korea*. Academy Publishing Company. 288 pp. [in Korean].
- LE PENNEC, M., P. G. BENINGER, G. DORANGE & Y. M. PAULET, 1991, Trophic sources and pathways to the developing gametes of *Pecten maximus* (Bivalvia: Pectinidae). *Journal of the Marine Biological Association of the United Kingdom*, 71: 451–463.
- LIM, H. K., C. S. GO & Y. H. LEE, 1995, Studies on the technology development for seed production of *Chlamys farreri*. Pp. 355–360, in: *Technical Report of South Sea Regional Fisheries*

- Research Institute, National Fisheries Research and Development Institute, Yosu, Korea.
- LIAO, C., Y. XU & Y. WANG, 1983, Reproductive cycle of the scallop *Chlamys farreri* (Jones and Preston) at Qingdao. *Journal of Fisheries of China*, 7: 1–13.
- MIN, D. K., J. S. LEE, D. B. KO & J. G. JE, 2004, *Mollusks in Korea*. Hanguel Graphics, Busan, Korea. 566 pp. [in Korean].
- MORVAN, C. & A. D. ANSELL, 1988, Streological methods applied to the reproductive cycle of *Tapes rhomboides*. *Marine Biology*, 97: 355–364.
- MOTAVKINE, P. A. & A. A. VARAKSINE, 1989, La reproduction chez les mollusques bivalves: rôle du système nerveux et regulation. *Reports Scientifiques et Techniques de l'IFREMER*, 10: 1–250.
- NA, G. H., W. G. JEONG & C. H. CHO, 1995, A study on seedling production of Jicon scallop, *Chlamys farreri*. 1. Spawning, development and rearing of larvae. *Journal of Aquaculture*, 8: 307–316.
- PIPE, R. K., 1987, Oogenesis in the marine mussel *Mytilus edulis*: an ultrastructural study. *Marine Biology*, 95: 405–414.
- SUN, H., S. KUANG & F. LI, 1996, Studies on suitable culture depths and method for scallop in Sanggou Bay. *Journal of Fisheries Science of China*, 3: 60–65.
- SUN, J., C. LIN, P. LI, Y. JIN & L. ZHOU, 1997, The culture experiment of scallop *Chlamys farreri* in Nanji Islands. *Zhejiang College of Fisheries*, 16: 247–255.
- WHANG, H. J. & M. N. KIM, 1973, Study on the distribution and ecology of *Chlamys farreri nipponensis* Kuroda around the Taehuksan Island. *Bulletin of National Fisheries Research and Development Agency*, 11: 25–35 [in Korean].
- WOURMS, J. P., 1987, Oogenesis. Pp. 50–157, in: A. C. GIESE, J. S. PEARSE & V. B. PEARSE, eds., *Reproduction of marine invertebrates*, Vol. 9. *General aspects: seeking unity in diversity*. Blackwell Scientific Publications, Palo Alto, California. xxii + 712 pp.
- YAKOVLEV, Y. M. & L. S. AFEICHUK, 1995, The reproductive cycle of the scallop *Chlamys farreri* in the Sea of Japan. Pp. 193–198, in: P. LUBET, J. BARRET & J.-C. DAO, eds., *Fisheries, biology and aquaculture of pectinids*, 8th International Pectinid Workshop, 273 pp.
- YANG, A., Q. WANG, J. KONG, P. LIU, Z. LIU, H. SUN, F. LI, R. WANG & M. JIANG, 1999a, Triploid induction in *Chlamys farreri* by application of 6-dimethyaminopurine. *Journal of Fisheries of China*, 23: 241–247.
- YANG, H., T. ZHANG, J. WANG, P. WANG, Y. HE & F. ZHANG, 1999b, Growth characteristics of *Chlamys farreri* and its relation with environmental factors in the intensive raft-culture areas of Sishiliwan Bay, Yantai. *Journal of Shellfish Research*, 18: 71–76.
- YOO, J. S., 1976, *Korean shells in colour*. Ilgisa, Seoul, Korea. 196 pp., 36 pp. pls. [in Korean].

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